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Defining the energy and nutrient content of corn grown in drought-stressed conditions and determining the relationship between energy content of corn and the response of growing pigs to xylanase supplementation

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Defining the energy and nutrient content of corn grown in drought-stressed conditions and determining the relationship between energy content of corn and the response of growing pigs to xylanase supplementation

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DEDICATION

To Emmett Rae and Finnegan Ricco, for your unconditional love and support during this entire process. Thank you for making me laugh each and every day. Without you two, I never would have made it.

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ABSTRACT

Two experiments were conducted to determine the effects of xylanase on apparent total tract-(ATTD) and apparent ileal digestibility (AID) in corn-based diets using higher and lower energy corn samples with pigs. The objective of these experiments was to determine if the efficacy of exogenous xylanases are increased in less digestible, as compared to more digestible, corn samples. First, corn samples of higher and lower digestible energy had to be identified. The 2012 drought-stressed growing conditions provided an opportunity to identify these samples. In experiment 1, 28 corn samples were selected from the 2012 crop, using yield as an initial screen for drought-stress, along with 2 samples from the 2011 crop to serve as controls. A total of 60 individually-housed barrows were randomly allotted to 1 of 30 diets, each containing 1 of the 30 corn samples, in a partial crossover design with 4 periods. Energy and nutrient content of corn grown in drought-stressed conditions (DS corn) was not different than what would have been expected in a typical year, but acid detergent fiber (ADF) and neutral detergent fiber (NDF) were higher in the DS corn than in the control corn ($P < 0.02$). Additionally, 2 corn samples of higher (HE) and 2 of lower (LE) digestible energy (3.74 and 3.75 Mcal DE/kg vs. 3.56 and 3.63 Mcal DE/kg) were identified in experiment 1 for use in a second experiment. In experiment 2, dietary treatments were arranged in a 2 x 2 factorial design: HE and LE corn samples, with and without xylanase supplementation; for a total of 8 dietary treatments. Sixteen individually-housed ileal-cannulated barrows were randomly allotted to 1 of the 8 diets in a 4-period partial crossover design. Mean ATTD and AID coefficients in the LE diets were not statistically different from

the HE diets with the inclusion of exogenous xylanase ($P > 0.10$). However, there was an overall effect showing an increase in ATTD of GE and DM with xylanase supplementation (84.8 vs. 83.6% for GE, $P = 0.008$; and 84.2 and 83.0%, $P = 0.007$). In conclusion, xylanase supplementation was not more effective in LE diets, as compared to HE diets, but there was an overall effect of enzyme increasing digestibility at the total tract level.

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Thesis Organization

This thesis was written to investigate the response of growing pigs to the inclusion of exogenous xylanase in corn-based diets. The first chapter gives a background on carbohydrates, carbohydrases, and previous studies related to this topic. It introduces the need for this research. The second and third chapters are derived from manuscripts that are in preparation for submission to the Journal of Animal Science. They are about two separate studies that aim to address a single question: Is the efficacy of exogenous xylanase increased in less digestible, as compared to more digestible, corn samples? The final chapter of this thesis consists of a summary and general conclusions from the two manuscripts. All references used throughout this thesis are located together at the very end.

Dietary Carbohydrates: Classifications and Definitions

Carbohydrates are naturally occurring compounds that consist of carbon, hydrogen, and oxygen in the ratio of C_n: H_{2n}: O_n (Benkeblia, 2014). They are the most common energy source in swine diets, comprising 60-70% of total energy intake (Bach Knudsen et al., 2012). Dietary carbohydrates can be classified according to their chemical properties, such as degree of polymerization, identity of individual monomers, and type(s) of glycosidic linkages present (Cummings and Steven, 2007). Dietary carbohydrates consist of monosaccharide units that are linked together to form disaccharides, oligosaccharides, or polysaccharides (Bach Knudsen et al., 2013). Monosaccharide units are linked together via glycosidic bonds.

These glycosidic bonds can be either α or ß, depending on the position of carbon atoms in the monosaccharides they link together. The type of linkages between monosaccharides can determine their fates within the body.

Polysaccharides are divided into two main groups: starch and nonstarch polysaccharides (NSPs; NRC, 2012). Both of these groups of polysaccharides are present in relatively large quantities in typical swine diets. However, starch is the main carbohydrate source in swine diets because it is the major storage carbohydrate in cereal grains (NRC, 2012). Starch is comprised entirely of glucose units that are linked together by α (1-4) or α (1-6) glycosidic bonds, and is digested and absorbed in the small intestine by endogenous enzymes. A portion of starch is not available for endogenous enzymatic digestion and absorption, and is known as resistant starch (Englyst et al., 1982). Resistant starches, gums, and pectins are all non-cell wall carbohydrates that are classified as NSPs. Carbohydrates that are not digested by endogenous enzymes of the pig are known as NSPs, or dietary fiber. Cellulose and hemicellulose are the most abundant NSPs in cell walls, however, other NSPs such as ß-glucans and arabinoxylans may also be present (Bach Knudsen, 2011). Cellulose is a linear, unbranched chain of glucose units bonded by ß (1-4) linkages, and hemicellulose is a branched-chain polysaccharide that is made up of numerous hexose and pentose units bonded by ß (1-4) linkages (NRC, 2012). Since both cellulose and hemicellulose contain ß glycosidic bonds, they cannot be digested in the small intestine of the pig. Carbohydrates that are not digested by the end of the small intestine must be either excreted in the feces or fermented in the hindgut via gut microbes (Bach Knudsen et al., 2012).

Carbohydrate Metabolism

Carbohydrate digestion and absorption

Carbohydrate digestion is initiated in the mouth when mixed with salivary amylase, but is relatively short because feed moves out of the mouth quickly, and salivary amylase is rapidly degraded in the stomach by the low pH environment (Englyst and Hudson, 2000). The majority of starch is digested in the small intestine via pancreatic and intestinal α-amylase and isomaltase, and maltase, which results in the release of glucose molecules (NRC, 2012). Starch digestion is very efficient, with over 95% occurring in the small intestine in most cereal grains used in swine diets (Bach Knudsen, 2001).

Only monosaccharides can be absorbed from the intestinal tract in monogastrics, and this only takes place in the small intestine. Therefore, digestive enzymes must hydrolyze the glycosidic bonds to free monosaccharides while they are still in the small intestine. Absorption of monosaccharides can occur via both passive and active transport through the enterocytes.

Fermentation of NSPs

The digestive enzymes of the pig are only capable of hydrolyzing certain types of glycosidic bonds. Therefore, many carbohydrates escape digestion in the small intestine. These carbohydrates may be fermented by microbes in either the small or large intestine (Jensen and Jorgensen, 1994), which results in the production of short-chain fatty acids (SCFAs); mainly acetic, propionic, and butyric (Macfarlane and Macfarlane, 1993). The SCFAs are absorbed and metabolized in the colonic epithelium, hepatic, fat, and muscle cells (Bergman, 1990), and can be used for energy or to synthesize adipose tissue (Bach Knudsen,

2001). Therefore, the SCFAs do contribute to the energy status of the pig; however, the energy contribution from SCFAs is not as efficient as that obtained from enzymatic hydrolysis of carbohydrates in the small intestine (Bach Knudsen, 2001). This makes fermentation less energetically favorable than enzymatic digestion and absorption of carbohydrates. Additionally, fermentation is much more variable than digestion (Bach Knudsen et al., 2008). The large variation in fermentability of fiber is attributed to differences in physicochemical properties including bulk, viscosity, solubility, and water-holding capacity (Zilstra et al., 2012). Some carbohydrates escape both enzymatic digestion and microbial fermentation, and therefore are excreted in the feces and do not contribute to the energy status of the pig.

Dietary Fiber Analysis

Many methods currently exist to measure dietary fiber in feedstuffs. One of the first methods developed and used was the crude fiber method, which is a gravimetric procedure (Henneberg and Stohmann, 1859). Due to the solubilization of the structural polysaccharides and lignin, this method incompletely and variably measures only certain fiber components (Bach Knudsen, 2001). In the 1960's, Van Soest developed the detergent methods for measuring fiber in feedstuffs (Van Soest, 1963; Van Soest et al., 1991; Van Soest and Wine, 1967). The detergent procedures allow for the determination of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL). The ADF procedure measures cellulose and lignin, and the NDF procedure measures hemicellulose, cellulose, and lignin, and the ADL procedure measures solely lignin. These methods allow for the individual determination of hemicellulose and cellulose by subtraction. There are however,

some concerns with the detergent method. For example, water-soluble NSPs or water-insoluble pectic substances are lost in the NDF procedure, starch and protein may contaminate the NDF portion, and hemicellulose may be left in the ADF portion after extraction (Bach Knudsen, 2001).

Recently, new methods for determining dietary fiber have been developed. One method is the enzymatic- and non-enzymatic gravimetric Association of Official Chemists procedures (Prosky et al., 1985). If the fiber is analyzed for total dietary fiber (TDF) using this method, both soluble and insoluble fractions can be measured. Another method is the enzymatic-chemical Englyst (Englyst et al., 1994) and Uppsala (Theander et al., 1994) procedures. These methods measure total soluble and total insoluble NSPs and their constituent sugars.

Fiber in the Diet

In growing pigs, digestibility coefficients of dietary fiber are approximately 0.40 – 0.50, which is significantly lower than other nutrients contained in feed, but this can range anywhere from 0 to 0.90, depending on fiber source (Noblet and Le Goff, 2001). This can cause problems in diet formulation, because different components of dietary fiber are digested differently by pigs. Due to lower digestibility, fiber inclusion in the diet is usually associated with a decrease in digestibility of the overall diet. Despite degree of digestion, fiber provides negligible amounts of digestible or metabolizable energy to growing pigs because it results in increased levels of endogenous protein and fat losses, as well as negative interactions between fiber and other dietary constituents (Noblet and Le Goff, 2001).

Dietary fiber is more favorable in adult pig diets than in diets for piglets or growing pigs. Research has shown greater digestibility of fiber and a greater content of metabolizable energy in sows than in growing pigs (Noblet and Shi, 1993; Jorgensen et al., 2007). Complex NSPs, such as arabinoxylans, are degraded to a much greater extent in sows than in growing pigs (Noblet and Bach Knudsen, 1997). Therefore, the use of technologies such as exogenous enzyme supplementation would appear to be much more beneficial in diets formulated for growing pigs.

The intake of dietary fiber has many physical effects on the pig. Due to relatively low levels of digestibility when compared with other nutrients, fiber increases fecal bulk and decreases transit time in the gastrointestinal tract. Insoluble, and thus, less-fermentable fiber are more resistant to microbial degradation than soluble fiber (Bach Knudsen et al., 2001).

Soluble vs. insoluble dietary fiber

Dietary fiber can be classified as soluble or insoluble, based upon its ability to interact with water (Kumar et al., 2012). Soluble dietary fiber causes more water-binding in the stomach and consequently reduces both satiety and digesta transit time. This can cause the formation of viscous gels, which entrap nutrients and hinder the ability of endogenous enzymes to access them for digestion and absorption. Increases in intestinal viscosity from soluble dietary fiber in swine diets have been shown to be closely related to a decrease in total tract digestibility of energy and other nutrients (Barrera et al., 2004). Additionally, increases in dietary fiber in swine diets have been shown to cause a decrease in apparent ileal digestibility of energy and other nutrients (Yin, et al., 2000). Insoluble dietary fiber, on the other hand, has little water-holding capacity and has been shown to decrease retention time in

the gastrointestinal tract, thus limiting the time that endogenous enzymes come into contact with the feed and therefore decreases nutrient digestion and fermentation (Bindelle et al., 2008). Insoluble dietary fiber is therefore known as a bulking agent.

Arabinoxylans

Arabinoxylans are the primary constituent of hemicelluloses. Although they can be present at highly variable concentrations, they have been shown at concentrations of about 5.2% in corn grain, and about 15.6% in corn co-products such as DDGS (Bach Knudsen, 1997). They are predominately comprised of two pentoses, xylose and arabinose, which is why they are commonly referred to as pentosans (Paloheimo, 2011). Their molecular structure consists of a linear main-chain comprised of (1-4)-ß-linked D-xylopyranose units which is substituted at the O2 and/or O3 positions of the xylosyl residues (Perlin, 1951). Other substituents, such as α-D-glucuronic acid and acetyl groups, can also be attached to the xylan backbone. Most arabinoxylans in cereal grains are anchored in the cells walls, which cause them to be insoluble (Mares and Stone, 1973). When not bound to the cell wall, arabinoxylans can absorb a great deal of water and form highly viscous solutions and therefore can result in increased viscosity of digesta (Choct, 1997 and Kumar et al., 2012).

Feruloylated arabinoxylans constitute a large portion of the cell walls in the aleurone layer, which contains lipids and proteins (Benamrouche et al., 2002). The endogenous enzymes of the pigs cannot hydrolyze the bonds that entrap these nutrients, thus making them unavailable to the pig. However, if the arabinoxylans can be broken down, it may cause these nutrients to become available for utilization by the pig. However, arabinoxylans require specific xylanases for degradation, which pigs are incapable of synthesizing, therefore

making the digestion of arabinoxylans in the gastrointestinal tract of the pig not possible. Certain gut microbes present in the pig's GI tract do, however, synthesize xylanases, therefore enabling arabinoxylans to be fermented to a certain extent in the pig's gut (Gdala et al., 1997 and Barrera et al., 2004).

Arabinoxylan metabolism

Complete hydrolyzation of arabinoxylans yields L-arabinose and D-xylose. In a study done by Wagh and Waibel (1967), it was shown that when chicks were injected with radioactive L-arabinose and D-xylose and the radioactivity in expired CO_2, liver glycogen, and excreta was measured, only a portion was recovered, which shows retention in the body. It was also shown that the weight gain of chicks was significantly suppressed when too much D-xylose or L-arabinose was substituted in the diet. This may indicate that, although they can be absorbed, D-xylose and L-arabinose are not well utilized by chicks. Though it has been shown that these molecules can be absorbed in the small intestine, the exact mechanism has yet to be elucidated (Schutte et al., 1991; 1992).

The apparent ileal digestibility of D-xylose and L- arabinose is 98.7, and 70.0% respectively, which shows that most disappears before the end of the small intestine (Schutte et al., 1991; 1992). However, it is unclear if the xylose and arabinose were absorbed or fermented in these studies because there was an increase in total SCFAs in the ileal digesta when they were included in the diet. This could indicate that some of the xylose and/or arabinose was fermented by microbes (Schutte et al., 1991; 1992). It is clear that additional research needs to be conducted to determine the fate of xylose and arabinose in monogastric animals.

Carbohydrases

Carbohydrases were developed in response to the issues associated with fiber in monogastric diets that consequently limit nutrient digestion. Xylanases and ß-glucanases are both largely produced carbohydrases that are currently being utilized in swine diets. By supplementing diets with these carbohydrases, it may be possible to shift a portion of fiber degradation from the hindgut to the small intestine. If this shift does occur, it could lead to an increase in the utilization of energy from feed ingredients that contain dietary fiber.

Although some performance benefits have been shown, the exact mode of action of xylanase is still unknown. There are, however, several theories that exist (Bedford, 2000). Viscous grains are often associated with increased intestinal viscosity, which slows down the rate of digestion, hindering access to their contents by digestive enzymes (Paloheimo et al., 2011). This can be seen through reduced feed conversion ratios and weight gain. Supplementation of carbohydrases in poultry diets have been shown to decrease viscosity and allow digestion to occur more rapidly and completely (Bedford, 2000), thereby improving animal performance. In more non-viscous grains, carbohydrase supplementation has been shown to reduce variation of feeding value and accelerate the rate of digestion (Bedford, 2000). It is thought that in these non-viscous grains, carbohydrases degrade plant cell walls, which leads to a release of nutrients such as amino acids, calcium, and phosphorus that are associated with the arabinoxylans from the grain endosperm and aleurone layer cells (Paloheimo et al., 2011). An additional proposed mechanism of carbohydrases is the prebiotic effect, which is achieved through the release of oligosaccharides (Choct and Cadogan, 2001). Oligosaccharides derived from cell wall digestion resist the attack of digestive enzymes, and are thus able to reach the colon to undergo fermentation. In the colon,

they have been shown to work as prebiotics to aid in the growth of beneficial microflora, which can suppress the growth of pathogenic microflora (Paleheimo et al., 2011).

In general, ß-glucanases are used in barley- and oat-based diets, and xylanases in wheat-based diets, due to their differing concentrations of specific NSPs. However, the use of these enzymes is expanding to inclusion in other cereal grain-based diets. These enzymes can be used alone, or in combination in animal diets.

The use of exogenous enzyme supplementation in poultry diets has become almost universal in Europe (Bedford, 2000), as the benefits have been clearly demonstrated in these diets. The addition of exogenous enzymes in poultry diets have been shown to increase the feeding value of ingredients (Claasen et al., 1985; Brenes et al., 1993, Bedford and Morgan, 1996; Steenfeldt et al., 1998), and reduce the variation in feeding value of ingredients (Scott et al., 1998; Bedford et al., 1998). The majority of research done with xylanase supplementation in swine diets to date has been with wheat-based diets because it is the major cereal grain used in Europe, which is where the enzyme was originally developed. In the United States, however, corn is the major cereal grain used in swine diets. Therefore, in order for xylanase to be successfully utilized in the United States, it must be shown that its supplementation can increase digestibility in swine diets containing corn and its co-products.

Carbohydrases in corn-based diets

Corn is generally referred to as a non-viscous cereal grain, containing a high proportion of highly digestible starch. Therefore, it was thought that its feeding value would not be enhanced by the addition of exogenous enzymes (Bedford, 1999). Many digestibility trials have measured feed intake and fecal output and assumed that the difference in nutrients

was of direct value to the animal. Nutrients may be released by microbes in the hindgut, but since hindgut fermentation is less energetically favorable to the pig, it is necessary to sample digesta at the terminal ileum via ileal cannulation trials as well as fecal output to measure fermentation by microbes.

Enzyme supplementation in corn-based swine diets have shown to provide benefits, such as improved digestibility of energy in some experiments (Myers et al., 2014; Fang et al., 2007), increased average daily gain (Fang et al., 2007), and feed conversion ratio (Fang et al., 2007). However, Olukosi et al. (2007) and Willamil et al. (2012) found no growth performance benefits in corn-based diets supplemented with xylanase. Additionally, Willamil et al. (2012) saw no digestibility improvements in the corn-based diets supplemented with xylanase. The efficacy of carbohydrases is variable, especially when supplemented in swine diets formulated with corn and its co-products (Jones et al., 2010; Yáñez et al., 2011). Further investigation of xylanase supplementation in corn-based diets fed to swine is necessary.

CHAPTER II

DEFINING THE ENERGY AND NUTRIENT CONTENT AND THE PHYSICAL PROPERTIES OF CORN GROWN IN DROUGHT-STRESSED CONDITIONS FED TO GROWING PIGS

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Abstract

Record-breaking heat and lack of rainfall during the 2012 growing season resulted in drought-stressed conditions in the U.S. corn-belt. The objective of this experiment was to investigate the impact of these conditions on nutrient composition and energy content in corn (DE, ME, and NE), and determine if relationships exist between corn quality measurements, nutrient content, and digestibility of energy. Twenty-eight samples of corn from the 2012 drought-stressed (DS) crop, plus 2 samples from the 2011 crop to serve as controls (CNTRL), were collected across Iowa and Illinois using yield as an initial screen for drought impact. Yields ranged from < 3.14 to > 12.55 t/ha. Each corn sample was graded by an official of the U.S. grain inspection agency and analyzed for 1,000 kernel weight, NIR values, total fat, starch, NDF, and CP content. Diets were formulated using each of the 30 corn samples and were fed at 2.6 times the estimated energy required for maintenance according to the 2012 NRC. Sixty individually-housed barrows (PIC 359 X C29; BW = 34.2 ± 0.2 kg) were randomly allotted in a partial crossover design with 30 diets and 4 periods. Diet and fecal samples were analyzed to determine DE values. ME and NE values were calculated from DE values using methods developed by Noblet and Perez (1993) and Noblet

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et al. (1994), respectively. Mean DE, ME, and NE values between the CNTRL and DS samples were not different (3.72 vs. 3.68 Mcal/kg respectively; 3.66 vs. 3.62 Mcal/kg respectively; and 2.92 vs 2.87 Mcal/kg, respectively). Comparing CNTRL with DS corn samples, there were no statistically significant differences ($P < 0.10$) in EE (4.07% vs 3.96%), CP (8.56 vs 9.18%), or starch (70.5 vs 69.5%). However, ADF and NDF were higher in the DS corn (2.23 and 8.19%) when compared to CNTRL (1.89 and 6.92%); $P < 0.001$ and $P = 0.0154$, respectively. Corn DE, ME and NE were lower than reported for corn by NRC (2012) and INRA (2002), but still within 2 standard deviations of the mean. DS corn samples contained less NDF, ADF and starch but similar CP and ether extract compared to NRC (2012) and INRA (2002). Weak but significant correlations were observed between DE and NDF ($R^2 = -0.26$; $P = 0.008$), kernel density ($R^2 = 0.26$; $P = 0.007$) and % damaged kernels ($R^2 = 0.17$; $P = 0.031$). We can conclude that corn grown in drought-stressed conditions was comparable in energy and nutrient concentration to non-drought-stressed corn and can be successfully utilized in swine diets.

Key words: pig, corn, drought, digestibility, energy

Introduction

Record-breaking heat and a lack of rainfall during the 2012 growing season resulted in drastically reduced yields and the expectation of a very poor quality corn crop. Severe stress from water deficiency during the growing period can result in yield loss through decreasing kernel mass and/or kernel number via abortion (Claassen and Shaw, 1970; Musick and Dusek, 1980). In addition, Westgate (1994) reported that both water deficiency and high temperatures shorten the kernel growth period. Premature termination of kernel

growth can lead to a large decrease in final endosperm and embryo mass (Ouattar et al., 1987; Grant et al., 1989; Westgate, 1994).

With ~712 million t produced annually (Markelz et al., 2011), corn is a common and widely used ingredient in swine diets. Corn is added to swine diets primarily as a source of energy. Variation in the concentration of energy in swine diets can lead to differences in growth performance and carcass composition (de la Llata et al., 2001; Beaulieu et al., 2009). When unusual cropping conditions occur, the industry logically asks if the nutritional value of corn is affected. In 2009, wet growing and harvest conditions resulted in lower nutrient content (Pilcher et al., 2011) and mold infestation (Patience et al., 2014). When the question was asked in relation to the 2012 crop year, the need for data on corn quality when grown under drought conditions was apparent, since there was a dearth of information on this subject.

The objectives of this experiment were to determine the impact of drought-stressed growing conditions on the energy content of corn and to determine if relationships exist among corn quality measurements, nutrient content, and energy digestibility. We hypothesized that corn grown in drought-stressed conditions would have lower energy content than corn grown in non-drought-stressed conditions.

Materials and Methods

All experimental procedures complied with the guidelines for the ethical and humane use of animals for research, and were approved by the Iowa State University Institutional Animal Care and Use Committee (#9-12-7441-S).

Animals, housing and experimental design

A total of 60 barrows (PIC 359 × C29; BW = 34.2 ± 0.2 kg) were randomly allotted in a partial crossover design with 30 diets and 4 periods. Through the 4 periods, no pig received the same diet twice. Each period consisted of 6 d of diet adaptation followed by 3 d of fecal collection. Pigs were fed a fully balanced grower diet (NRC, 2012) for 5 d between each period to alleviate any negative effects associated with being on a protein deficient diet for an extended period of time.

Pigs were housed individually in 1.0 x 1.8 m pens. Each pen had a partially slatted concrete floor, individual feeder, and nipple drinker. Water was provided *ad libitum* throughout the experiment. Test diets were fed at 2.6 times the estimated energy required for maintenance (NRC, 2012) based upon the average weight of the pigs at the beginning of each replicate period. Feed allowance was divided into equal rations that were fed in mash form twice daily at 08:00 and 16:00.

Diets and feeding

A total of 38 corn samples were collected across Iowa and Illinois using yield as an initial screen for drought-stress. It was assumed that samples obtained from lower yielding fields were affected most by drought. Corn samples were collected as evenly as possible within the following predetermined yield (t/ha) categories: < 6.28, 6.28 to 7.85, 7.86 to 9.42, 9.43 to 10.98, and > 10.99. All yield categories were selected to be below yields typically expected for corn grown in Iowa and Illinois, with the assumption that all corn samples would therefore be drought-stressed. Since corn samples obtained from lower yielding fields were assumed to be affected most by drought stress, the goal of the yield categories was to

obtain samples that were increasingly affected by the drought. All samples were tested by the Iowa State University Veterinary Diagnostic Laboratory (Ames, IA 50011) for aflatoxin contamination, and any samples exceeding 20 ppb were discarded. Additionally, each sample was graded by an official U.S. grain inspection agent for moisture content, test weight, total and heat-damaged kernels, and broken and foreign material. The samples were then tested at Iowa State University for 1,000 kernel weight and density. Two control (CNTRL) corn sample treatments from the 2011 crop and 28 drought-stressed (DS) corn sample treatments from the 2012 crop were selected for use in this experiment based on yield category and absence of aflatoxin.

Thirty diets were formulated using each of the previously mentioned corn samples, vitamins, minerals, and 0.4% titanium dioxide as an indigestible marker (Table 2.1) to determine apparent total tract digestibility (ATTD) of energy. Vitamins and minerals were supplied at levels formulated to meet or exceed nutrient requirements of growing pigs (NRC, 2012). Other than minor amounts supplied from the vitamin premix, the only source of energy in the diets was corn, the ingredient of interest. Corn was ground in a hammer mill to a mean particle size of 647 microns.

Sample collection and analysis

Fresh fecal samples were collected on d 7, 8, and 9 of each time period via grab sampling. Pens were scraped on d 6 to ensure fresh sample collection. Representative corn and diet samples were collected at the time of feed mixing and stored at - 20°C until analyzed.

Fecal samples were thawed at room temperature, homogenized, dried in a forced air oven at 65°C (Yamato Mechanical Convection Oven DKN810), and ground through a 1.0 mm screen (Wiley Mill 3379-K35, Thomas Scientific, Swedesboro, NJ) prior to analysis. DM of feed and feces was determined by drying at 105°C to a constant weight (method 967.03; AOAC, 1990). GE of feed and feces was determined using an isoperibolic bomb calorimeter (Model 6200, Parr Instrument, Moline, IL). Benzoic acid was the standard used to calibrate the instrument (6,318 kcal/kg actual; 6,320 ± 5 kcal/kg determined). Titanium dioxide content in both the feed and feces was determined according to Leone (1973).

Corn samples were processed and analyzed in the same manner as feed samples. Corn samples were additionally analyzed for nitrogen using a TruMac®N Nitrogen Analyzer (Leco Corporation, St. Louis, MO) according to method 990.03 (AOAC, 2007), with EDTA as a standard used to calibrate the instrument. Total starch content was determined using a commercially available kit (Megazyme K-TSTA, Wicklow, Ireland) following modified method 996.11 (AOAC, 2007). Both ADF and NDF were quantified using an Ankom Fiber Analyzer (Model 2000, ANKOM Technology Method 9, Ankom Technology, Macedon, NY). Total fat was determined following acid hydrolysis using ether extraction according to method 920.39 (AOAC, 2007).

All analyses were carried out in duplicate, except NDF which was assayed in triplicate. Analyses were repeated when the intra-sample CV exceeded 3%.

Apparent total tract digestibility coefficients (ATTD) were calculated for GE and DM according to Oresanya et al. (2007). DE was calculated as gross energy measured in the corn times the ATTD of GE. ME and NE values were calculated from DE using the equations of Noblet and Perez (1993) and Noblet et al. (1994), respectively.

Statistical analysis

This experiment was designed as a partial crossover design with 30 dietary corn treatments and 4 periods. The PROC UNIVARIATE procedure (Version 9.3, SAS Inst., Cary, NC) was used to verify normality and homogeneity of variances of the variables, and all data were analyzed using the PROC MIXED procedure of SAS. Individual animal and corn sample were the experimental units for analyzing data from the digestibility trial and analyses of the chemical constituents, respectively. The model for ATTD of energy and DM included the fixed effect of treatment and random effect of replicate period. Differences between least squares means were separated using the PDIFF option of SAS with results considered significant if P was ≤ 0.05 and trends if P was > 0.05 and ≤ 0.10. Correlation coefficients were determined using the PROC CORR procedure of SAS and are reported as Pearson coefficients. Correlations were considered significant if P was ≤ 0.05 and trends if P was > 0.05 and ≤ 0.10.

Results and Discussion

Comparison of corn samples harvested in 2011 (CNTRL) with drought-stressed 2012 samples (DS) provides some basis for yr-to-yr differences, especially since all samples were handled identically and evaluated under the same experimental conditions. However, with only 2 samples included from the former yr, conclusions drawn from the comparison must be interpreted with great care. Selection of samples was based on the study objective, which was to characterize corn grown under drought-stressed conditions, not to compare the two yr directly.

There were few differences between CNTRL and DS corn samples in terms of physical characteristics (Table 2.2). While the kernels in the DS corn appeared visually smaller than the CNTRL corn, the determination of mean 1,000 kernel weight revealed no differences ($P > 0.10$).

The most variable characteristics of corn grain used in this experiment were 1,000 kernel weight and yield (120% and 505%, respectively; Table 2.2), which was likely caused by the variation in degree of drought-stress experienced by each sample based upon growing location. Water deprivation has been shown to cause premature termination of the grain fill period, thus causing decreased kernel weight and overall yield (Prasad and Staggenborg, 2008). Differences in the degree of drought-stress among samples would likely cause variation in length of the grain fill period, and therefore would explain the differences observed in kernel sizes. Carbohydrate reserves in the leaves and stalks, in addition to nitrogen reserves in the leaves, can be mobilized to aid in nutrient deposition in kernels during times of stress (Westgate, 1994). When compared with values reported in the NRC (2012), all nutrient concentrations from the corn in this experiment were within, or close to, expected ranges. It is likely that due to drought-stress, the plant mobilized more carbohydrate and nitrogen stores from the leaves and stalk to aid in kernel growth than it would under normal conditions. This would result in the normal nutrient concentrations that were observed in combination with small kernel sizes due to early termination of the grain fill period.

Despite this wide variation in kernel weight, the variation in average kernel density among samples only ranged from 1.26 to 1.30 g/cc with a mean of 1.27 g/cc. There tended to

be a higher proportion of damaged kernels in DS corn ($P = 0.068$). Samples of DS corn ranged from 2.45 t/ha to 14.81 t/ha, meeting our study objectives.

The DS corn samples had higher NDF concentrations compared to CNTRL samples (8.19 vs. 6.92%, $P = 0.015$; Table 2.4). INRA (2002) and NRC (2012) reported slightly higher NDF content at 12.0% and 10.3%, respectively, suggesting that fiber content of corn grown in the DS crop yr was not abnormally high. Additionally, DS samples had higher ADF concentrations than CNTRL samples (2.23 vs. 1.89%, $P < 0.001$, Table 2.4). No other differences in chemical constituents of the corn samples were detected, despite a numerical increase in CP (9.2 vs 8.6%) and a numerical decrease in starch (69.5 vs 70.5%) in DS versus CNTRL ($P > 0.10$). Elevated levels of CP would be expected in corn grown under drought-stress conditions (Oktem, 2008).

The mean CP level observed in DS was similar to the values reported by the NRC (9.3%; 2012), Feedstuffs (8.7%; 2014) and INRA (9.4%; 2002) for yellow dent corn. Cromwell et al. (1999) reported substantial variation in the composition of 45 corn samples collected over 3 yr. They reported CP values of 9.6, 9.2, and 8.9% for 1989, 1990 and 1992, respectively, further suggesting that the DS corn samples used in this experiment were not unusual in their protein content. The 4.0% ether extract reported in Table 2.4 is also similar to the 4.3, 4.2, and 4.1% reported by INRA (2002), NRC (2012) and Feedstuffs (2014), respectively. Finally, the starch content of the DS corn (69.5%) was only slightly less than the 74.2% reported by INRA (2002) and the 70.8% reported by NRC (2012). Overall, data did not suggest that DS corn was much different than typical corn, in terms of chemical composition.

The GE of corn would not be expected to differ among yr, unless the fat content also varied. Since ether extract values were virtually identical to CNTRL samples, no differences in GE were expected or observed ($P > 0.10$; Table 2.4). The ATTD of DM was different between CNTRL (84.4%) and DS (83.4%; $P < 0.001$), and while a numerical difference in ATTD of GE was observed (84.3 and 83.1% for CNTRL and DS, respectively), it was not different ($P > 0.10$). At least part of the difference in statistical precision between DM and energy can be explained by the large difference in the SEMs, 0.20 for ATTD of DM and 0.81 for ATTD of GE.

Similarly, there were no differences between the CNTRL and DS corn samples for DE, ME or NE (Table 2.4; $P > 0.10$). The ME and NE values were calculated from DE using generally accepted equations: Noblet and Perez (1993) for ME and Noblet et al. (1994) for NE. The range in DE values, at about 8%, is typical of energy measurements in swine (Jacobs et al., 2013).

The DE content of CNTRL (3.72 Mcal/kg DM) and DS samples (3.68 Mcal/kg DM) were below the 3.91 Mcal/kg DM reported by NRC (2012), the 3.93 Mcal/kg DM reported by INRA (2002) and the 3.90 Mcal/kg DM reported by Feedstuffs (2014). The ME content of CNTRL (3.66 Mcal/kg DM) and DS samples (3.62 Mcal/kg DM) were below the 3.84 Mcal/kg DM reported by the NRC (2012), 3.85 Mcal/kg DM reported by INRA (2002), and 3.90 Mcal/kg DM reported in Feedstuffs (2014). The NE content of CNTRL (2.92 Mcal/kg DM) and DS samples (2.87 Mcal/kg DM) were also lower than the 3.03 Mcal/kg DM reported by the NRC (2012) and the 3.07 Mcal/kg DM reported by INRA (2002). Nothing in the chemical composition of the CNTRL or DS corn samples explains the lower energy content published in common databases. However, values reported herein fall within 2

standard deviations of the means reported by the NRC (2012), suggesting they fall within normal values expected for corn.

No relationships were observed between any single corn quality measurement, physical or chemical, and the DE content of the corn samples (Figures 2.1 to 2.11). It was surprising, however, that no relationship was apparent between corn yield and DE content. According to the experimental hypothesis, a decrease in crop yield, which served as a proxy for degree of drought stress, would be associated with a decrease in corn energy content. The data are clear in this regard (Figure 2.1), and it is unlikely that a larger sample size would elicit any different conclusion, as a large range in yield was observed without a relationship to DE. It is possible that yield was not a suitable proxy for drought stress, or perhaps modern hybrids are capable of maintaining nutritive value in spite of severe water inadequacy.

It was not expected that test weight would correlate with corn DE (Figure 2.2). This has been observed in other crops, such as barley (Fairbairn et al., 1999) and wheat (Zijlstra et al., 1999). The poor relationship between bulk density of corn and energy content has also been demonstrated in poultry (Leeson et al., 1993; Dale, 1994). As a result, it was not surprising there was no correlation between 1,000 kernel weight and DE content (Figure 2.3).

A positive correlation was observed between kernel density and DE; as density increased, so too did DE (Figure 2.4; $P = 0.007$; $R^2 = 0.26$). Additionally, there was a positive correlation between total damaged kernels and DE (Figure 2.5; $P = 0.031$; $R^2 = 0.17$). It is difficult to understand this relationship, since the opposite was observed in corn during the very wet 2009 crop yr (Pilcher et al., 2011). No relationship was observed between broken kernels and foreign material and energy content (Figure 2.6). There was no correlation between CP (Figure 2.7) and energy content.

Surprisingly, no relationship existed between fat content of the corn and DE content, as some samples with the lowest fat content (< 3.5%) had among the highest DE content (> 3.7 Mcal/kg; Figure 2.8). Similarly, there was no correlation between corn starch content and DE (Figure 2.9). Some samples with the highest starch content (> 60.5%) had among the lowest DE values (< 3.6 Mcal/kg).

There was a negative correlation between NDF and DE, such that as fiber increased, DE decreased (Figure 2.11; $P = 0.008$; $R^2 = -0.26$). This relationship is rational, since higher fiber levels would be associated with lower digestibility of GE (Gutierrez et al., 2013). However, this relationship was not observed between ADF and DE (Figure 2.10).

Overall, corn grown in drought-stressed conditions was comparable in energy and nutrient concentration to corn grown in a "normal" yr. Despite lower quality measurements, such as yield or 1,000 kernel weight, feeding values appeared to remain unaffected. Therefore, corn grown under drought-stressed conditions can be successfully utilized in swine diets.

Table 2.1. Ingredient composition of test diets, % as-fed basis

Item	
Corn	96.54
Limestone	1.23
Monocalcium phosphate	1.19
Salt	0.40
Vitamin premix[1]	0.14
Trace mineral premix[2]	0.10
Titanium dioxide	0.40

[1]Provided per kilogram of complete diet: vitamin A, 6,614 IU; vitamin D, 827 IU; vitamin E, 26 IU; vitamin K, 2.6 mg; niacin, 29.8 mg; pantothenic acid, 16.5 mg; riboflavin, 5.0 mg; vitamin B_{12}, 0.023 mg.
[2]Provided per kilogram of diet: Zn, 165 mg as zinc sulfate; Fe, 165 mg as iron sulfate; Mn, 39 mg as manganese sulfate; Cu, 17 mg as copper sulfate; I, 0.3 mg as calcium iodate; and Se, 0.3 mg as sodium selenite.

Table 2.2. Physical characteristics of corn samples used in diet formulation[1]

Item	CNTRL[2]	DS[3]	DS Range	SEM	*P*-value
N	2	28	--	--	--
Yield, t/ha	--	7.97	2.45 – 14.81	--	--
Test weight, kg/hL	73.9	73.1	69.0 – 76.0	1.87	0.653
1000 kernel weight, g	337	284	176 – 386	55.8	0.344
Density, g/cc	1.27	1.27	1.26 – 1.30	0.024	0.904
Total damaged kernels, %	0.9	1.7	0.2 – 7.9	0.40	0.068
Broken kernels and foreign material %	0.8	0.7	0.2 – 2.0	0.66	0.953
Particle size, microns	625	647	525 – 844	59.5	0.718

[1]All values presented on an as-is basis
[2]CNTRL = control samples from 2011 crop; collected from Ames, IA
[3]DS = samples grown in drought-stressed conditions from 2012 crop across Iowa and Illinois

Table 2.3. Physical characteristics of DS[1] corn samples by yield category (t/ha)[2]

Item	< 6.28	6.29-7.85	7.86-9.42	9.43-10.98	>10.99	Pooled SEM	*P*-value
N	8	3	7	4	5	--	--
Test weight, kg/hL	73.0	72.8	72.8	73.1	73.6	0.89	0.933
1000 kernel weight, g	268	272	266	305	325	20.4	0.119
Density, g/cc	1.27	1.25	1.26	1.26	1.28	0.010	0.414
TDK[3], %	2.4	1.8	2.2	1.1	0.4	0.57	0.001
BKFM[4], %	0.9	1.0	0.8	0.3	0.4	0.18	0.004
Particle size, microns	692	651	642	582	636	29.5	0.103

[1]DS = samples grown in drought-stressed conditions from 2012 crop across Iowa and Illinois
[2]All values presented on an as-is basis
[3]TDK = total damaged kernels
[4]BKFM = broken kernels and foreign material

Table 2.4. Chemical composition of corn samples used in diet formulation[1]

Item	CNTRL[2]	DS[3]	DS range	SEM	*P*-value
N	2	28	--	--	--
CP, %	8.56	9.18	7.98 – 11.07	0.379	0.108
Total fat, %	4.07	3.96	2.91 – 4.83	0.183	0.579
Starch, %	70.5	69.5	67.4 – 71.6	1.21	0.419
NDF, %	6.92	8.19	7.02 – 10.14	0.489	0.015
ADF, %	1.89	2.23	1.82 – 3.14	0.073	<0.001

[1]All values presented on a dry-matter basis
[2]CNTRL = control samples from 2011 crop; collected from Ames, IA
[3]DS = samples grown in drought-stressed conditions from 2012 crop across Iowa and Illinois

Table 2.5. Chemical composition of DS[1] corn samples by yield category (t/ha)[2]

Item	<6.28	6.29-7.85	7.86-9.42	9.43-10.98	>10.99	Pooled SEM	*P*-value
N	8	3	7	4	5	--	--
CP, %	9.53	9.29	9.29	8.75	8.65	0.292	0.122
Fat, %	4.12	3.93	3.80	4.32	3.72	0.155	0.076
Starch, %	69.6	68.6	69.6	69.0	70.2	0.53	0.408
NDF, %	8.38	8.14	8.29	8.84	7.22	0.372	0.006
ADF, %	2.35	2.18	2.23	2.15	2.13	0.111	0.530

[1]DS = samples grown in drought-stressed conditions from 2012 crop across Iowa and Illinois
[2]All values presented on a dry-matter basis

Table 2.6. Digestibility and energy content of corn samples used in diet formulation[1]

Item	CNTRL[2]	DS[3]	DS Range	Pooled SEM	*P*-value
N	2	28	--	--	--
DM, %	89.41	89.79	86.3 – 92.3	0.352	0.280
GE, Mcal/kg	4.42	4.43	4.40 – 4.49	0.007	0.116
ATTD of DM	84.4	83.4	81.4 – 85.0	0.20	<0.001
ATTD of GE	84.3	83.1	80.6 – 85.6	0.81	0.150
DE, Mcal/kg	3.72	3.68	3.54 – 3.82	0.042	0.359
ME, Mcal/kg[4]	3.66	3.62	3.48 – 3.75	0.041	0.299
NE, Mcal/kg[5]	2.92	2.87	2.76 – 2.97	0.031	0.160

[1]All values reported on a dry-matter basis
[2]CNTRL = control samples from 2011 crop; collected from Ames, IA
[3]DS = samples grown in drought-stressed conditions from 2012 crop across Iowa and Illinois
[4]Calculated using Noblet and Perez (1993)
[5]Calculated using Noblet *et al.* (1994)

Table 2.7. Digestibility and energy content of DS[1] corn samples by yield category (t/ha)[2]

Item	< 6.28	6.29-7.85	7.86-9.42	9.43-10.98	>10.99	Pooled SEM	*P*-value
N	8	3	7	4	5	--	--
DM, %	88.69	90.42	89.75	91.01	89.77	0.648	0.041
GE, Mcal/kg	4.45	4.43	4.42	4.43	4.40	0.048	<0.001
ATTD of DM	83.1	83.7	83.3	82.8	84.2	0.36	0.092
ATTD of GE	83.1	83.3	83.1	82.3	83.9	0.49	0.292
DE, Mcal/kg	3.70	3.69	3.67	3.65	3.69	0.028	0.683
ME, Mcal/kg[3]	3.63	3.63	3.61	3.59	3.63	0.027	0.718
NE, Mcal/kg[4]	2.88	2.87	2.86	2.86	2.88	0.021	0.825

[1]DS = samples grown in drought-stressed conditions from 2012 crop across Iowa and Illinois
[2]All values reported on a dry-matter basis
[3]Calculated using Noblet and Perez (1993)
[4]Calculated using Noblet *et al.* (1994)

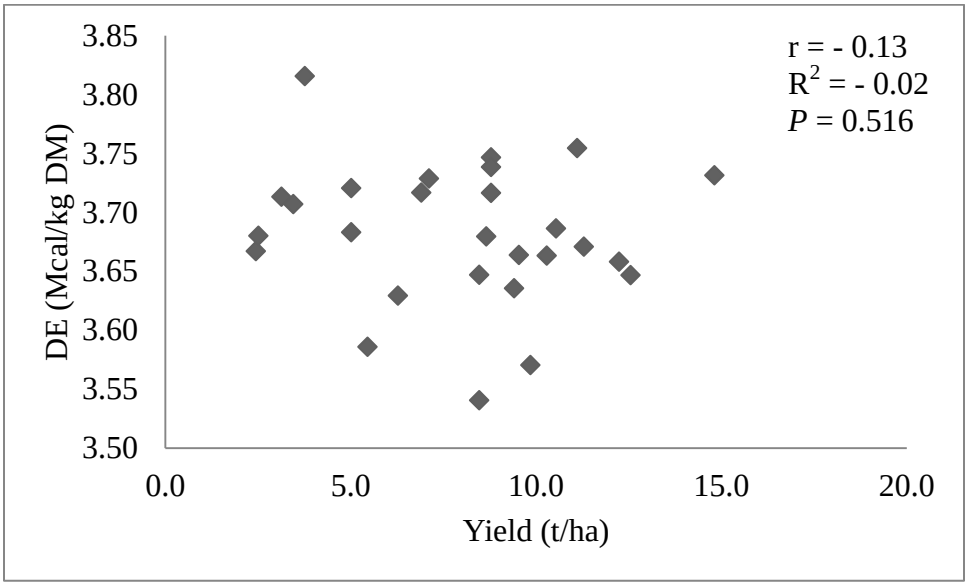

Figure 2.1. Pearson correlation between DE and yield in 2012 drought-stressed corn samples

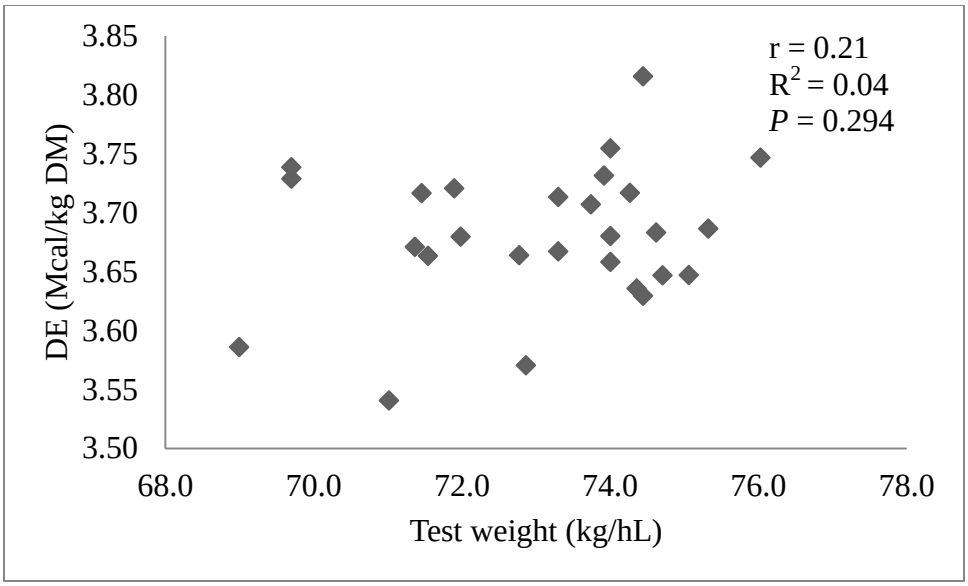

Figure 2.2. Pearson correlation between DE and test weight in 2012 drought-stressed corn samples

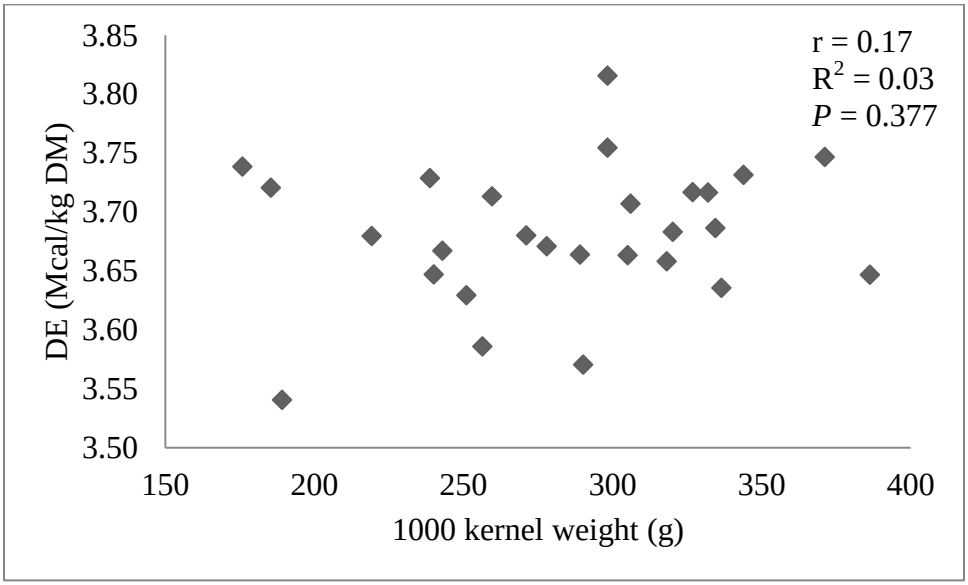

Figure 2.3. Pearson correlation between DE and 1000 kernel weight in 2012 drought-stressed corn samples

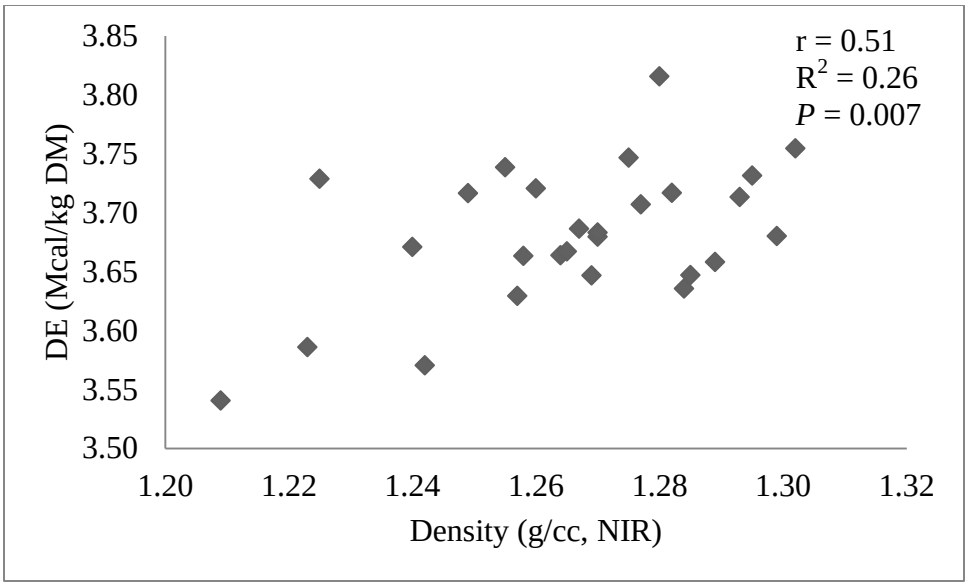

Figure 2.4. Pearson correlation between DE and NIR density in 2012 drought-stressed corn samples

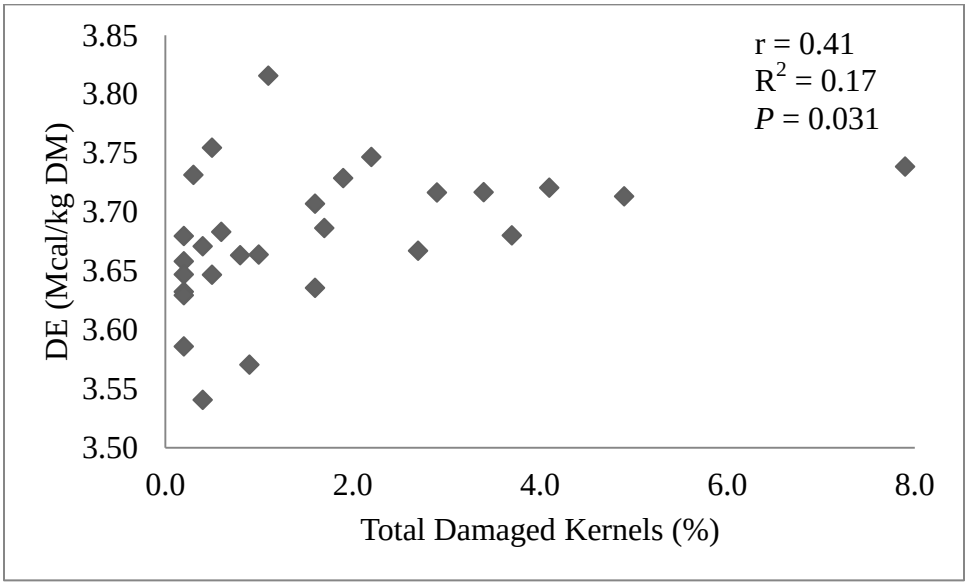

Figure 2.5. Pearson correlation between DE and TDK in 2012 drought-stressed corn samples

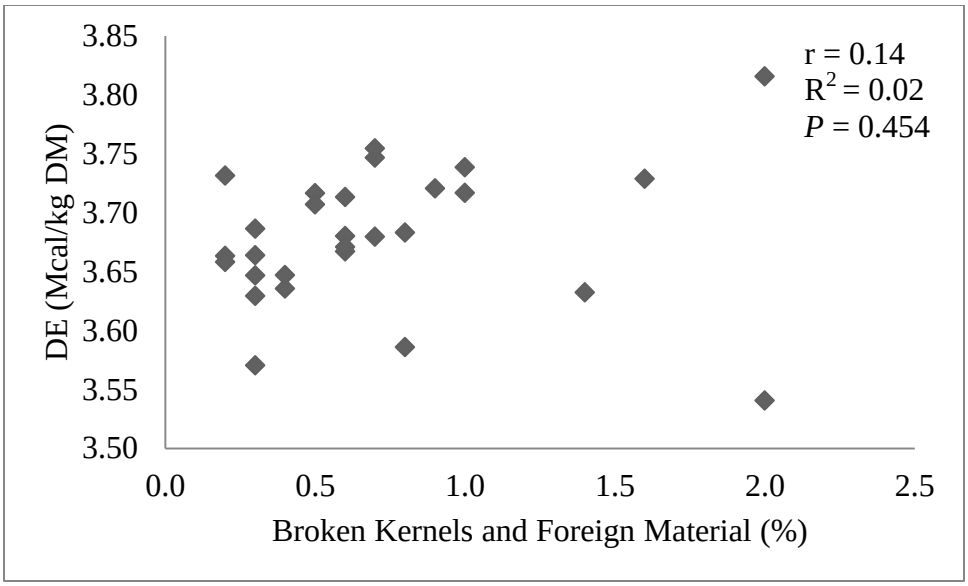

Figure 2.6. Pearson correlation between DE and BKFM in 2012 drought-stressed corn samples

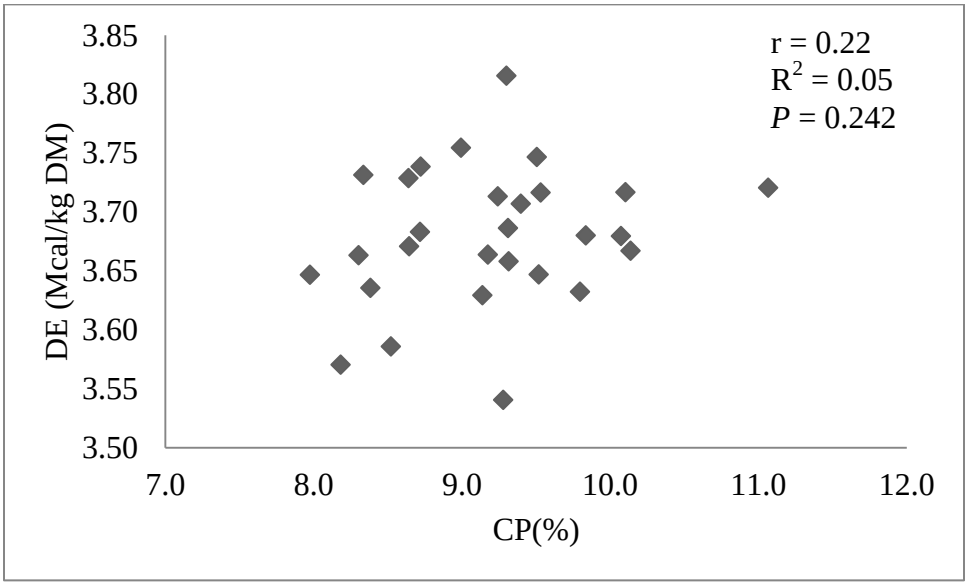

Figure 2.7. Pearson correlation between DE and CP in 2012 drought-stressed corn samples

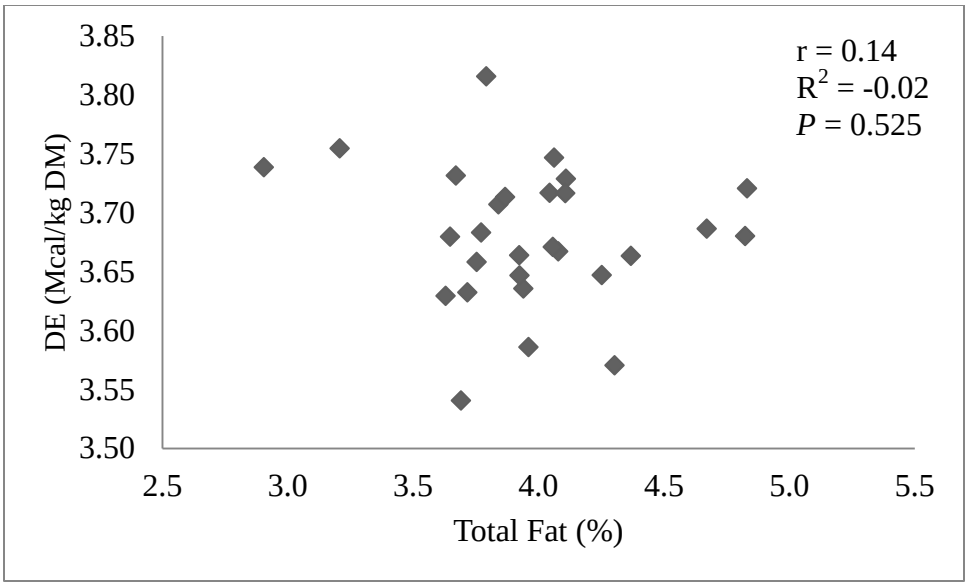

Figure 2.8. Pearson correlation between DE and total fat in 2012 drought-stressed corn samples

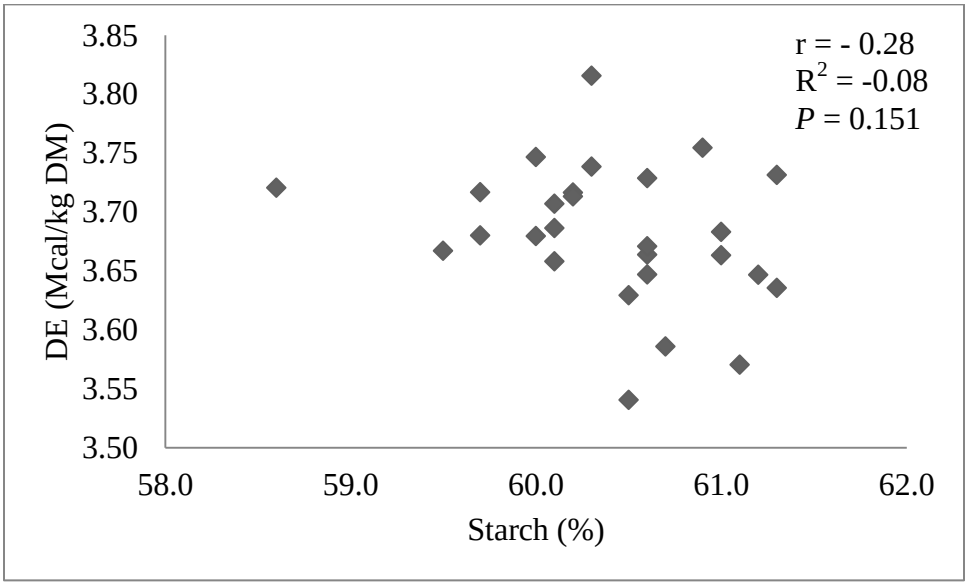

Figure 2.9. Pearson correlation between DE and starch in 2012 drought-stressed corn samples

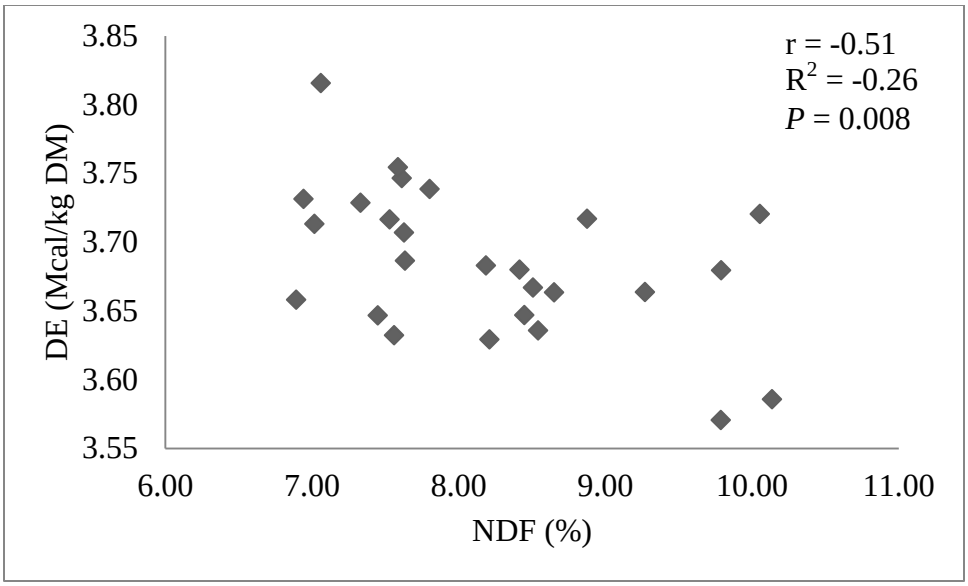

Figure 2.10. Pearson correlation between DE and NDF in 2012 drought-stressed corn samples

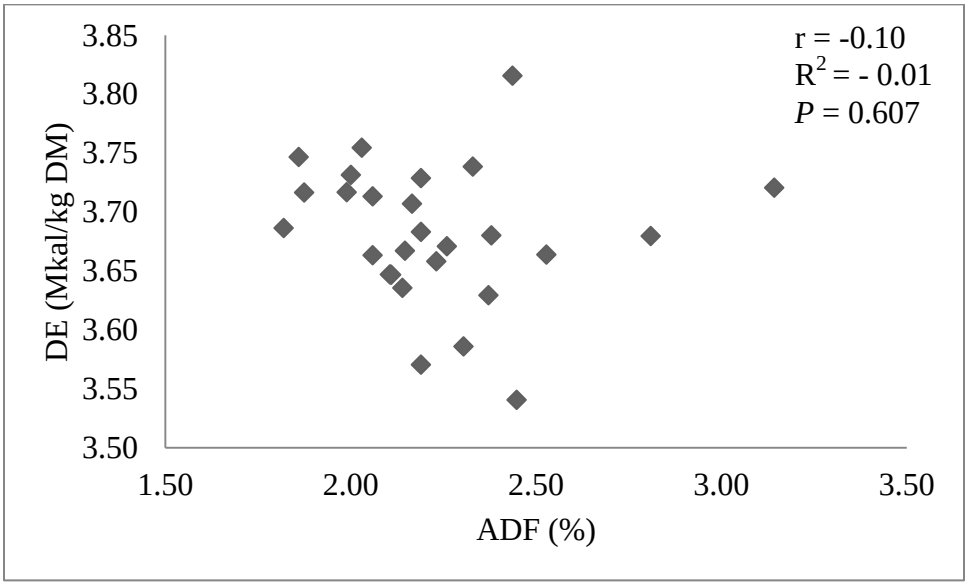

Figure 2.11. Pearson correlation between DE and ADF in 2012 drought-stressed corn samples

CHAPTER III

THE RELATIONSHIP BETWEEN THE ENERGY CONTENT OF CORN AND THE RESPONSE OF GROWING PIGS TO EXOGENOUS XYLANASE SUPPLEMENTATION.

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Abstract

The overall objective of this experiment was to determine if the efficacy of exogenous xylanase is increased in less digestible, as compared to more digestible, corn samples. Four corn samples, 2 with higher (HE; 3.75 and 3.74 Mcals/kg) and 2 with lower (LE; 3.63 and 3.56 Mcals/kg) DE values, were selected based upon determination in a previous digestibility trial. Diets were formulated using each 4 corn sample, casein, vitamins, minerals, and 0.4% chromic oxide as an indigestible marker. Each of the 4 diets were then arranged in a 2 x 2 factorial design: high DE and low DE, with and without xylanase supplementation; for a total of 8 dietary treatments. Diets were fed at a level of approximately 3 times the estimated energy required for maintenance (NRC, 2012) based on average initial BW of pigs at the start of each replicate period. Each replicate period consisted of 9 d adaptation to diets followed by 2 d of fecal and then 3 d of ileal collection. Sixteen individually housed barrows (PIC 359 X C29; initial BW=34.8±0.23kg) were randomly allotted to treatment in a 4-period partial crossover design. Diet, fecal, and ileal samples were analyzed in order to determine apparent total tract (ATTD) and apparent ileal digestibility (AID) coefficients. Mean ATTD of GE and AID coefficients in the LE diets were

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not statistically different from the HE diets with inclusion of exogenous xylanase. However, there was an overall effect of ATTD of GE and DM with enzyme supplementation (84.8 vs. 83.6% for GE with and without enzyme, respectively, $P = 0.008$; and 84.2 and 83.0% with and without enzyme, respectively, $P = 0.007$). In conclusion, the addition of the xylanase enzyme was not more effective in the LE diets, as compared to the HE diets, but there was an overall effect of enzyme addition increasing total tract digestibility.

Key words: pig, xylanase, carbohydrase, digestibility, energy

Introduction

Corn remains the primary ingredient used in U.S. swine diets. Its main source of energy is starch with lessor contributions from protein and fat. It contains a relatively small quantity of non-starch polysaccharides (NSP; Newman et al., unpublished data). Yet, Gutierrez et al. (2014) reported that the concentration of xylose, a sugar representing only 3% of the grain and found largely in the insoluble NSP fraction, can explain about 70% of the variation in energy content among corn co-products. Corn is considered relatively uniform, although the standard deviation for DE content is estimated at 111 kcal/kg (NRC, 2012), or about 3% of the mean. Because dietary energy concentration is a major determinant of pig growth performance (Beaulieu et al., 2009), and also is the single most costly dietary constituent by volume, even small variation in the concentration of energy in corn is problematic. Technologies that reduce this variation would be of great interest and value to the pork industry.

The NSPs in corn are not utilized well by swine because they lack the proper digestive enzymes (Gutierrez et al., 2013). Since they cannot be digested in the pig, NSPs

and associated nutrients are primarily digested via microbial fermentation (Li et al., 1996). This process is less efficient in regards to energy utilization than enzymatic digestion (Noblet et al., 1994).

Supplementation of exogenous enzymes such as xylanase may increase degradation of NSP, although the exact mechanism has not yet been elucidated. If carbohydrases increase degradation of constituents of fiber, and if corn samples vary in the quantity of fiber, it can be hypothesized that response of pigs to exogenous xylanase will be greater in lower quality, higher fiber corn, as compared to higher quality, lower fiber samples. Therefore, the objective of this experiment was to determine if the efficacy of exogenous xylanase is increased in less digestible, as compared to more digestible, corn samples.

Materials and Methods

All experimental procedures adhered to the ethical and humane use of animals for research, and were approved by the Iowa State University Institutional Animal Care and Use Committee (#2-13-7511-S).

Animals, housing, and experimental design

The experiment was conducted in an environmentally controlled room at the Iowa State University Swine Nutrition Farm. A total of 16 crossbred barrows (PIC 337 x C22/C29; initial BW = 34.8 ± 0.23 kg) were surgically fitted with a T-cannula inserted in the distal ileum to allow for collection of ileal digesta (Stein et al., 1998). After surgery, pigs were housed individually in 1.8 × 1.9 m pens. Each pen had a partially slatted concrete floor, a

stainless steel feeder, and a nipple drinker. Water was provided *ad libitum* throughout the experiment.

During the 10-d recovery period after surgery, pigs consumed to appetite a corn-soybean meal-based diet that was formulated to meet or exceed swine nutrient requirements (NRC, 2012). Following recovery, pigs were randomly allotted to one of 8 dietary treatments in 4 replicate periods each lasting 14 d. Each replicate period consisted of 9 d of diet adaptation followed by 2 d of fecal collections and then 3 d of ileal collection. This provided a total of 8 observations per dietary treatment.

Pigs were weighed and randomly allotted to dietary treatments the evening before the first d of each replicate period. Care was taken to ensure no pig was allotted to the same diet twice during the total experimental period.

Diets and feeding

Eight corn samples (4 higher in DE and 4 lower in DE) were pre-selected for DE values based on a previous trial (Newman et al., unpublished data). Within these 8 corn samples, those with the most similar DE values were paired and pooled together for each experimental diet, resulting in four corn samples for use in this experiment. Two of these diets were then higher in DE content (HE; 3.74 and 3.75 Mcal DE/kg) and 2 were lower in DE content (LE; 3.63 and 3.56 Mcal DE/kg). The HE diets are referred to as HE1 and HE2, and the LE diets are referred to as LE1 and LE2. The analyzed nutrient composition of the corn samples is presented in Table 3.1.

Each of the 4 corn samples were thus arranged in a 2 x 2 factorial arrangement, with energy level (high versus low) versus enzyme (with versus without) as the factors. The

xylanase product (Econase XT 25P, AB Vista, Marlborough, England) was added at 100 g/tonne provided 1600 BXU/kg in the final diet.

Experimental diets were fed at 3.0 times the estimated energy requirement for maintenance (NRC, 2012) based on average weight of pigs at the beginning of each replicate period and were divided into equal rations that were fed twice daily at 07:00 and 15:00.

Experimental diets consisted of one of four corn samples plus casein, with vitamins and minerals added to meet or exceed requirements for 30 kg pigs (NRC, 2012). Chromic oxide (0.4%) was included in the diets as an indigestible marker (Table 3.2). The only source of carbohydrates in the diets was from corn, the ingredient of interest. Diets were mixed in a small mixer, and ingredients other than corn and casein were premixed in a dough mixer to ensure thorough mixing and achieve a uniform final product. The formulated nutrient concentration in the diets is presented in Table 3.2.

Sample collection

Pens were scraped on d 9 and fresh fecal samples were collected on d 10 and 11 via grab sampling. Samples were immediately stored at -20°C until later processing for assay. Ileal samples were collected on d 12, 13, and 14 for 8 h each d by attaching a 207-mL plastic bag (Whirl-Pak, Nasco, Fort Atkinson, WI) to the opened cannula with a cable tie and catching natural flow. Bags were removed when they were full with digesta or at least every 30 min. All fecal and digesta samples were immediately stored at -20°C for later processing and assay.

Analytical methods and calculations

At the conclusion of the experiment, ileal and fecal samples were thawed at room temperature and mixed thoroughly within animal and replicate period. A sub-sample was collected and stored at -20°C for later assay.

Digesta samples were lyophilized (Model 10-100, Virtis Co. Ltd., Gardiner, NY) and ground through a 1-mm screen (Wiley Mill 3379-K35, Thomas Scientific, Swedesboro, NJ) prior to chemical analyses. Fecal samples were dried in a forced air oven at 65°C (Yamato Mechanical Convection Oven DKN810, Yamato Scientific America Inc., Santa Clara, CA) and then ground through a 1.0 mm screen (Wiley Mill 3379-K35, Thomas Scientific, Swedesboro, NJ) prior to analyses. Diet samples were also homogenized and ground prior to analyses.

Feed, fecal and digesta samples were stored in desiccators and analyzed in duplicate at the Iowa State University Monogastric and Comparative Nutrition Laboratory (Ames, IA). DM was determined by drying at 105˚C to a constant weight (method 967.03; AOAC, 1990). GE of feed, fecal, and digesta samples was determined using an isoperibolic bomb calorimeter (Model 6200, Parr Instrument Co, Moline, IL). Benzoic acid was the standard used to calibrate the instrument (6,318 kcal/kg actual; 6,323 ± 0.65 kcal/kg determined). Chromic oxide was determined using the method of Fenton and Fenton (1979); absorption was measured at 440 nm using a spectrophotometer (Synergy 4, BioTek, Winooski, VT). Chromic oxide standard samples were assayed to confirm the accuracy of the analytical procedure, and a recovery of 100.9 ± 1.7% was attained.

The AID and ATTD of dietary components were calculated using the following equation (Oresanya et al., 2007):

AID or ATTD, % = 100 – [100 × (concentration of Cr_2O_3 in diet × concentration of component in feces or digesta/concentration of Cr_2O_3 in feces or digesta × concentration of component in diet)].

Hindgut fermentation was calculated using difference, as shown in the following equation:

ATTD, % - AID, % = Hindgut fermentation, %.

The amount of DM (g/kg DMI) or GE (Mcal/kg DMI) remaining at the terminal ileum and excreted in feces were calculated using the following equations:

Amount remaining at terminal ileum (g or Mcal/kg DMI) = [Concentration of component in digesta × (Cr_2O_3 in diet / Cr_2O_3 in digesta)]

Amount excreted in feces (g or Mcal/kg DMI) = [Concentration of component in feces × (Cr_2O_3 in diet / Cr_2O_3 in feces)]

Disappearance of DM (g/kg DMI) and GE (Mcal/kg DMI) prior to the terminal ileum and in the hindgut were calculated using the following equations:

Disappearance prior to the terminal ileum = [intake – amount remaining at terminal ileum]

Hindgut disappearance = [amount remaining at terminal ileum – amount excreted in feces]

Statistical analysis

The PROC UNIVARIATE procedure (SAS Inst., Cary, NC) was used to verify normality and homogeneity of variances of the variables, and all data were analyzed using the PROC MIXED procedure of SAS (Version 9.3, Cary, NC). The individual pig served as

the experimental unit for all analyses. The model included the fixed effects of DE content (high vs. low), enzyme inclusion (with vs. without), and their interaction. Random effects included replicate period, pig, and corn sample (sample 1 vs. 2 of the HE samples, and sample 1 vs. 2 of the LE samples). Differences between least squares means were separated using the PDIFF option of SAS with results considered significant if P was ≤ 0.05 and trends with P-values between 0.05 and 0.10.

Results

Pigs remained healthy during the experiment. Health was monitored daily and illness was determined by appetite, lethargy, gauntness, breathing regularity, gait and/or cannula function. Additionally, all pigs consumed their daily feed allowance throughout the experiment. The BW of pigs at the start of periods 1, 2, 3, and 4 was 34.8 ± 0.2, 43.8 ± 0.4, 54.5 ± 0.5, and 66.5 ± 0.5 kg, respectively. Corn samples used in this experiment were selected for higher or lower DE levels, based upon determination in a previous experiment (Newman et al., unpublished data). The HE corn samples had approximately 150 kcals more DE than the LE corn samples (3740 and 3750 vs. 3630 and 3560 kcals/kg, respectively). On average, the LE corn contained 27% higher ADF than the HE corn (2.32 and 2.60% vs. 2.15 and 1.71%, respectively). The LE corn also contained approximately 27% higher NDF content than the HE corn (9.71 and 10.07% vs. 7.93 and 7.66%, respectively).

Digestibility of DM and GE

The AID of GE and DM were not affected by diet or inclusion of xylanase in the diet, and there were no diet × enzyme interactions (Table 3.2). The release of GE and DM in the

hindgut were not affected by inclusion of xylanase in the diet, and there were no diet x enzyme interactions (Table 3.3), but there was a trend for release of GE in the hindgut to be higher in the HE corn than the LE corn (5.5 and 5.6% for HE vs. 2.8 and 3.8% for LE; P = 0.092). Over the total tract, digestibility was higher in the HE samples (84.4 and 85.7% for GE; 83.5 and 84.8% for DM) than the LE samples (83.1 and 83.6% for GE; 82.8 and 83.3% for DM); P = 0.008 for GE and 0.011 for DM. Additionally, enzyme inclusion increased overall ATTD of GE (84.8 vs 83.6%; P < 0.001) and DM (84.2 vs 83.0%; P = 0.007). However, there were no diet x enzyme interactions.

Flow of DM and GE

There was no diet x enzyme interaction in flow, disappearance, or excretion of DM or GE (Table 3.4). Pigs fed diets formulated with the HE corn samples had greater hindgut disappearance of DM than those fed diets formulated with the LE corn samples (180 and 186 vs. 149 and 150 g/kg DMI respectively, P = 0.031), but had similar flow through the terminal ileum. Pigs fed diets formulated with HE corn samples also tended to excrete lower amounts of GE in the feces than those fed diets formulated with the LE corn samples (0.70 and 0.63 vs. 0.75 and 0.72 Mcal/kg DMI respectively, P = 0.076), though disappearance and flow of GE through the intestinal tract was similar.

Pigs fed diets without xylanase inclusion excreted greater amounts of DM in the feces than those fed diets with xylanase (136 g/kg and 151 g/kg of DMI, P = 0.024). The pigs fed diets without xylanase also excreted greater amounts of GE in the feces compared to those fed diets with xylanase (0.74 vs. 0.66 Mcal/kg DMI, P = 0.013). Flow of GE and DM through the intestinal tract was similar between pigs fed diets with and without xylanase,

although pigs fed diets with xylanase tended to have lower amounts of GE remaining at the terminal ileum (0.87 vs. 0.94 Mcal/kg DMI, $P = 0.089$).

Discussion

Though corn is considered relatively uniform, its fiber content has been shown to vary quite a bit relative to its concentration in the grain (Newman et al., unpublished data; NRC, 2012). Corn samples used in this study were selected to have differing levels of DE and ME. Diets with high fiber content usually contain less ME than diets with low fiber content (Wenk, 2001). Therefore, it was not surprising that the difference in energy content resulted in differing NDF content between samples, with the HE samples averaging 7.8% and the LE samples averaging 9.9%. Enzyme supplementation has been shown to reduce variation in ingredient quality (Bedford, 2000). In this experiment, enzyme supplementation did decrease the coefficient of variance of ATTD of GE among the corn samples by 0.2%, but this was a relatively small effect.

Carbohydrase supplementation in diets formulated with viscous cereal grains, such as wheat or barley, has been shown to decrease intestinal viscosity and thus the rate of digestion in these diets, at least in poultry (Paloheimo, 2011). The NSP portion of corn mainly consists of insoluble arabinoxylans (Summers, 2001) and at low levels (about 5.2%; Bach Knudsen, 1997), so xylanase is not expected to impact intestinal viscosity as much as would be the case in the more viscous grains that contain higher concentrations of soluble NSP (Evers et al., 1999). Though it has been shown to have performance benefits, the exact mechanism of action of exogenous xylanase is still unknown (Bedford, 2000).

Enzyme supplementation has been shown to be most effective when supplemented in poor quality cereal grains, at least in poultry (Bedford et al., 1998; Choct et al., 1995). So, it was surprising that the LE corn samples did not have a greater response to enzyme supplementation than HE corn samples. Enzyme supplementation results in improvements in nutrient extraction in the small intestine, which consequently results in reduced microbial activity through substrate limitation in the ileum (Bedford, 2000). This did not appear to be the case in this experiment. Though AID of GE and DM numerically improved with the addition of xylanase, the improvement was small and was not statistically significant. We can infer that the effect of enzyme supplementation in swine may differ from that of poultry, probably due to large differences in gastrointestinal physiology and function.

Interestingly, there tended to be an effect of corn quality on hindgut fermentation, such that greater quantities of GE were released in the large intestine in the HE, as compared to the LE, samples. (5.6 and 5.5% vs. 2.8 and 3.8% for HE and LE respectively; $P = 0.092$). As discussed previously, the HE samples had lower ADF and NDF concentrations. Increased fiber would mean an increase in substrate for potential fermentation by microbes in the hindgut, so it is of great interest that the HE samples were fermented to a greater extent than the LE samples. The level and composition of dietary fiber can cause differences in digestion and fermentation (Wenk, 2001); therefore, looking at the dietary fiber level alone cannot completely indicate its influence on digestion and fermentation. Perhaps evaluating the samples for specific NSP levels or viscosity would help to understand the differences in fermentation among corn samples. We did not measure viscosity or levels of specific NSP present in each corn sample used in this experiment. Although it may be helpful in explaining the unexpected differences in fermentation between LE and HE samples, it would not help to

accomplish the objective of this study, which was to define the effect of enzyme in the HE vs. LE corn samples.

The ATTD of GE and DM were improved with the addition of xylanase (83.6 vs. 84.8% for GE, $P < 0.001$; and 83.0 vs. 84.2% for DM, $P = 0.007$), which has also been shown in other studies (Myers et al., 2014; Fang et al., 2007), although xylanase supplementation had no effect on AID or lower gut release of GE and DM. It is likely that in this study, the effect of enzyme was additive, and therefore was only identifiable across the total tract.

No differences were observed in DM or GE flow through the intestinal tract. However, the main effects of both diet and enzyme had an effect on the excretion of DM and GE in the feces. In all cases, xylanase supplementation decreased the energy excreted in the feces. This suggests that xylanase promoted additional energy release, and is supported by the increase observed in ATTD of GE with enzyme supplementation across diets.

This study was unable to support the hypothesis carbohydrases have greater impact with poor quality grains when compared to higher quality grains Overall, xylanase supplementation proved to increase ATTD of GE and DM, but did not elicit a greater response in less digestible samples than in the more highly digestible samples in this experiment. These results may be due to the relatively low concentrations of arabinoxylans (about 5.2%; Bach Knudsen, 1997) in corn grain, which would mean less substrate for the xylanase to act on, and a small or undetectable response.

Table 3.1 Nutrient composition of 2 higher energy (HE) and 2 lower energy (LE) corn samples[1]

	HE-1	HE-2	LE-1	LE-2
DE, Mcal/kg	3.75	3.74	3.63	3.56
ADF, %	2.15	1.71	2.32	2.60
NDF, %	7.93	7.66	9.71	10.07

[1]All values are reported on a DM basis, analyzed values from use in previous trial

Table 3.2 Ingredient and nutrient composition (as-fed basis) of experimental diets[1]

Ingredients, %	
Corn[2]	83.76
Casein	12.70
Monocalcium P	1.30
Limestone	1.20
Salt	0.40
Vitamin Premix[3]	0.14
Trace Mineral Premix[4]	0.10
Chromic Oxide	0.40
Nutrients, calculated, %	
ADF	1.92
NDF	7.73
SID Lysine[5]	1.00
Calcium	0.72
STTD P[6]	0.41

[1]Econase XT 25P was added to 4 of the 8 diets at 100 g/tonne
[2]4 different corn samples used, 2 with higher DE and 2 with lower DE according to previous data
[3]Provided per kilogram of complete diet: vitamin A, 6,614 IU; vitamin D, 827 IU; vitamin E, 26 IU; vitamin K, 2.6 mg; niacin, 29.8 mg; pantothenic acid, 16.5 mg; riboflavin, 5.0 mg; vitamin B12, 0.023 mg.
[4]Provided per kilogram of diet: Zn, 165 mg as zinc sulfate; Fe, 165 mg as iron sulfate; Mn, 39 mg as manganese sulfate; Cu, 17 mg as copper sulfate; I, 0.3 mg as calcium iodate; and Se, 0.3 mg as sodium selenite.
[5]SID lysine = Standardized ileal digestible lysine
[6]STTD P = Standardized total tract digestible phosphorus

Table 3.3 Comparison of apparent ileal (AID) and total tract digestibility (ATTD), and hindgut fermentation in 2 higher energy (HE) and 2 lower energy (LE) corn samples with and without the addition of Econase XT[1]

	HE-1		HE-2		LE-1		LE-2			*P*-value		
Item, %	+	-	+	-	+	-	+	-	SEM	Diet	Enzyme	D x E[2]
AID												
GE	79.5	78.7	80.5	79.9	80.8	79.6	80.1	79.2	1.63	0.744	0.311	0.996
DM	77.8	77.2	78.3	78.1	79.3	78.4	78.7	78.3	1.70	0.686	0.522	0.994
HF[3]												
GE	6.5	4.7	5.6	5.3	2.9	2.7	3.2	4.4	1.80	0.092	0.737	0.702
DM	7.3	5.3	6.9	6.1	3.9	3.5	3.8	5.2	1.90	0.113	0.678	0.666
ATTD												
GE	85.4	83.4	86.0	85.3	84.0	82.3	83.8	83.4	0.86	<0.001	0.008	0.436
DM	84.4	82.5	85.3	84.4	83.7	81.9	83.2	83.3	0.85	0.007	0.011	0.299

[1] Econase XT 25P was added at 100 g/tonne

[2]D x E = diet by enzyme interaction

3HF = Hindgut fermentation = (total tract digestibility – apparent ileal digestibility)

Table 3.4 The effects of diet, enzyme, and diet by enzyme on flow of DM and GE of experimental diets formulated with 2 higher energy (HE) and 2 lower energy (LE) corn samples with and without the addition of Econase XT[1]

	HE-1		HE-2		LE-1		LE-2		Pooled	*P*-value		
Item	+	-	+	-	+	-	+	-	SEM	Diet	Enzyme	D x E[2]
Intake												
DM, kg/d	1.65	1.64	1.63	1.63	1.66	1.66	1.68	1.69	-	-	-	-
GE, Mcal/d	7.39	7.39	7.29	7.32	7.37	7.44	7.49	7.53	-	-	-	-
DM, g/kg DMI												
Disappearance prior to TI[3]	676	674	689	679	713	687	708	697	13.1	0.128	0.205	0.852
Remaining at TI	324	326	311	321	287	313	292	303	13.1	0.128	0.205	0.852
Hindgut disappearance[5]	189	171	190	181	146	152	145	155	15.3	0.031	0.774	0.779
Excreted in feces[6]	135	155	121	140	141	161	147	148	8.7	0.141	0.024	0.707
GE, Mcal/kg DMI												
Intake	4.49	4.50	4.48	4.48	4.46	4.49	4.45	4.47	-	-	-	-
Disappearance prior to TI[3]	3.53	3.54	3.64	3.58	3.66	3.55	3.58	3.50	0.065	0.522	0.172	0.826
Remaining at TI	0.96	0.96	0.84	0.90	0.80	0.94	0.87	0.97	0.064	0.441	0.089	0.743
Hindgut disappearance[5]	0.31	0.21	0.25	0.23	0.10	0.14	0.17	0.24	0.073	0.256	0.950	0.644
Excreted in feces[6]	0.65	0.75	0.59	0.67	0.70	0.80	0.70	0.73	0.043	0.076	0.013	0.832

[1] Econase XT 25P was added at 100 g/tonne

[2]D x E = diet by enzyme interaction

[3]Disappearance prior to the terminal ileum (TI) = [intake – amount remaining at terminal ileum]

[4]Amount remaining at terminal ileum = [component in digesta × (Cr_2O_3 in diet / Cr_2O_3 in digesta)]

[5]Hindgut disappearance = [amount remaining at terminal ileum – amount excreted in feces]

[6]Amount excreted in feces = [component in feces × (Cr_2O_3 in diet / Cr_2O_3 in feces)

CHAPTER IV

SUMMARY AND CONCLUSIONS

Enzyme supplementation has been a large focus in farm animal diets over the past few decades. Great success has been demonstrated with phytases, and with carbohydrases in poultry diets. However, use of xylanase in swine diets, especially with non-viscous grains, has shown more inconsistent responses. This thesis focused on quantifying the feeding value of corn grown in drought conditions and the response of growing barrows to xylanase supplementation in corn-based diets of differing energy levels.

Unlike corn grown in wet conditions, corn grown in extremely dry conditions is relatively similar in nutritive value to swine when compared to a typical yr, which was a novel finding. This was surprising because yields and seed weights were extremely variable, ranging from 2.45 to 14.81 t/ha and 176 to 386 g, respectively. However, these physical characteristics appear to have no effect on feeding value of the grain to swine.

Though many benefits have been shown with xylanase supplementation in poultry, this thesis demonstrates that similar digestibility benefits might not be replicated in barrows, likely due to their differing digestive physiology. However, the overall increase in apparent total tract digestibility with xylanase supplementation implies potential use for this enzyme in swine diets and indicates need for future research. Since the mode of action of xylanase in non-viscous grains has not yet been elucidated, more research needs to be conducted investigating its mode of action to determine how to supplement corn-based diets effectively. If successful, xylanases could be supplemented more widely in commercial swine diets in the U.S.

In this study, we did not measure arabinoxylan concentration in the diets. Since substrate variability influences the response to exogenous enzyme supplementation, knowing the concentration of the substrate would be beneficial. We originally made the assumption that less digestible corn would elicit a greater response with enzyme addition because we assumed that less digestible corn samples would contain more arabinoxylans. The less digestible samples did have a higher concentration of fiber; however, there simply may not be enough substrate for the enzyme to elicit a significant response. The ability to measure specific NSP may have improved our understanding of the results.

Flow and digestibility data for total dietary fiber would have been extremely advantageous in this thesis to further explain some of the results seen. Unfortunately, the Monogastric and Comparative Nutrition laboratory's LECO was down for months before this thesis needed to be published, so the complete total dietary fiber data is not included here. However, this data will be included when the individual manuscript is submitted to the Journal of Animal Science.

To conclude, enzyme supplementation in animal diets has been successful in the past, and has promise for the future. In order to utilize each enzyme most effectively it is necessary to first discover their mode of action to determine how best to utilize them in different grain-based diets, and when fed to different species.

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